Physical aspects of incubation and its relation with embryonic development

By R. Meijerhof

The earliest reference to artificial incubation can be found in Aristotle's Historia Animalium, written in the 4th century BC, and it is known that the ancient Greeks and Chinese were already practicing artificial incubation succesfully. Already at that time, it was known that for successful incubation, the environment around the egg needs to be controlled. During incubation, the eggs need to be turned, and temperature, humidity and ventilation need to be controlled. When the correct conditions are applied to the egg, the embryo will start to develop and will grow into a complete chick, ready for hatching. The process of turning the content of the egg into a hatchling is from a biological standpoint extremely complicated, and far from being fully understood. However, the physical conditions that control the biological process are relatively easy to understand and describe.

The environment around the egg influences embryonic development and hatchability (Ognabesan et al, 2007). During incubation, the embryo consumes oxygen and produces carbon dioxide, water and heat (Romanoff, 1967). Robertson (1923) already stated that the speed of the complex process of growth and development will be determined by the slowest component. As gas exchange and temperature determine the exchange of oxygen, water carbon dioxide and heat, this means that these physical conditions are determining the developmental processes. Temperature is recognized to be the most important climatical condition (Romanoff, 1960; Deeming and Fergusson, 1991, Decuypere and Michels, 1992), as relatively small differentiations from the optimum will quickly influence the process and with it the quality and the survival rate of the embryos. As the biological processes involved in development and grow require oxygen and produce carbon dioxide, these gases have to pass the membranes and the egg shell during the incubation process. During the incubation period also metabolic water is produced, that needs to leave the egg to create an air cell to allow the embryo to develop long respiration prior to the start of the hatching process. The three different processes and their physical requirements (temperature, oxygen and carbon dioxide exchange and moisture loss) will be discussed in the following section.

Temperature

Temperature is by far the most critical factor during incubation (Lundy, 1969; Wilson, 1991). Many experiments and field results have shown that a differences of fractions of a degree Celsius in air temperature will influence embryonic development (Romanoff 1960), hatchability (Wilson, 1991), navel quality (Lourens et al, 2005, 2007; Hulet et al, 2007) and post hatch performance (Lundy 1969, Wilson 1991). Not only organ weight and then especially heart weight is influenced by temperature (Wineland et al., 2000; Leksrisompong et al., 2007; Lourens et al., 2007), but also metabolic disorders as ascites (Molenaar et al, 2011) bone development (Oviedo-Rondón et al., 2009) and immune system (Durant et al, 2012, Walstra et al, 2012) are influenced by temperature during incubation. However, in recent years it has become clear that the determining factor is not the temperature of the air, but the temperature of the embryo inside the shell. This is by itself logical, as the embryo will react on the temperature outside of the shell by the influence it will have on the temperature of its direct environment, which is the temperature inside of the shell.

In natural incubation conditions, the embryo uses the gradient in temperature between the brood path of the mother hen and the bottom of the nest, by actively directing blood flow to regulate its

internal temperature, as shown by Tzschentke and Nicolmann (1997). By directing the blood flow from its body to a larger or smaller extent to the relative cooler or warmer part of the egg, it can influence its core body temperature. This allows the embryo in natural incubation to be less dependent on temperature conditions outside of the egg shell, but the same authors also shown that the embryo reacts already on slight differences in internal temperature, indicating that it tries to regulate temperature in a very narrow range.

In artificial incubation, we provide the eggs with a uniform temperature around the egg. This means that the embryo cannot regulate its own internal temperature as it has no gradient in temperature that will help to regulate its temperature if the direction of the blood flow is changing. This means that the control of the internal egg temperature has to be done completely from the outside of the egg, by the control of the incubation conditions.

Embryo temperature

Embryo temperature is the core body temperature of the embryo inside the egg. Although this temperature is not completely uniform but fluctuates slightly between the different parts of the body, for practical reasons we can assume that the temperature distribution between the different parts of the body of the embryo is uniform. As the egg shell loses heat, there is a temperature gradient between the body of the embryo and the shell of the egg. Meijerhof and van Beek (1993) presented a model which can be used to calculate this gradient, based on the thermal properties of the content of the egg and the theory of heat diffusion. Although the thermal properties of yolk and albumen are not completely equal (Meijerhof and van Beek, 1993), the differences are marginal. If we assume that the temperature of the embryo is uniformly distributed over its body, it means that the gradient between embryo and shell will be a function of the initial difference in temperature between embryo and shell, the thermal properties of the egg content, and the amount of egg content between embryo and shell.

For practical reasons, egg shell temperature is often used as an indicator for embryo temperature. Measuring the egg shell temperature is not invasive, and when done with modern infrared equipment, is much more quick then measuring embryo temperature which requires a probe inside the egg. However, it must be realized that in some situations egg shell temperature and embryo temperature can be substantially different. Although extensive studies involving the relation between egg shell temperature and embryo temperature are not to the authors knowledge, some assumptions in this respect can be made.

Egg shell temperature vs embryo temperature

At the start of incubation, there is a lot of egg content between the embryo and the egg shell. The embryo is not producing any metabolic heat yet, which means that an equilibrium will be reached at which the temperature of the egg is uniform and constant. However, during the warming process that equilibrium is not reached yet, as the egg shell will be warmed much more quickly than the embryo itself, and will therefore be substantially warmer than the embryo. Measuring the egg shell as an indicator for embryo temperature is in this situation of limited use, as the difference between embryo temperature and egg shell temperature will be high.

Once heat production of the embryo starts, the embryo will be warmer than the surrounding air and heat will be transported from the embryo to the shell, creating again a gradient. This gradient is again depending on the thermal properties of the egg and the amount of heat being produced, but as the embryo is developing blood vessels towards the egg shell, the actual heat transport will probably be bigger than can be expected from just the thermal properties of the egg content. In the first half of the incubation process the amount of blood vessels will be minimal and the distance between the embryo and the shell surface will be large, but as the heat production of the embryo is also still minimum, a high difference between embryo and egg shell is not to be expected.

In the second half of the incubation process the embryonic heat production increases dramatically, which will increase the gradient in temperature between the embryo and the surface of the egg shell. However, as the embryo is also increasing in size and has developed a significant blood vessel system under the shell, the heat transfer from embryo to egg shell will be increased as well, suggesting that the temperature gradient between embryo and egg shell surface will be limited.

This means that although embryo temperature and egg shell temperature are not identical, for practical reasons egg shell temperature can be used as an indicator for embryo temperature, unless rapid temperature changes are applied. This is especially the case during the warming period of the eggs, in which the egg shell temperature will go up much more quickly than the temperature of the embryo itself.

Balance in heat

Several authors (Owen, 1991; Meijerhof and van Beek, 1993; French, 1997) developed models to calculate the influence of the environment on the temperature of an egg. Although the models differ in complexity, the main principle is that the temperature of an egg during incubation is a balance between heat production (negative and positive) and heat transfer from the egg to the environment of vice versa.

The heat transfer (the cooling and warming process of eggs) is basically influenced by two factors, the heat flow through the egg content and heat transfer between egg shell and surrounding air, which is mainly a function of differences in temperature between the egg shell and the air and air velocity over the egg shell, (Meijerhof and van Beek, 1993). These authors presented a mathematical model that quantifies the physical aspects involved. Lourens et al (2010) compared the results of measurements with the calculated values from this model, and found a correlation between calculated and observed values for temperature of eggs of 0.99 for small eggs and 0.98 for large eggs. This indicates that the actual temperature of eggs can be calculated accurately when the conditions are known.

Negative heat production

Eggs constantly lose water through the pores. This water is evaporated from the egg shell and creates a cooling effect, which acts a negative heat production. The amount of water that is lost in a specific time period is determined by the conductance of the egg shell and the difference in water vapor pressure (the water vapor pressure deficit) across the egg shell (Meijerhof and van Beek, 1993). Water vapor pressure is the result of temperature and relative humidity, and will therefore be

influenced by the environmental conditions. The cooling effect of the moisture loss can be determined by the evaporative heat loss of water, and the specific heat of eggs.

The energy that is required to evaporate water is 2.25kJ/g. This means that when 1 gram of water is evaporated from the egg shell, the egg will lose 2.25kJ of heat. This has a cooling effect and in a steady-state situation, the resulting temperature of the egg will decrease. As the specific heat of eggs is approximately 3.31 kJ/kg/oC (Romanoff and Romanoff, 1949, as cited by Turner, 1985), an egg of 60 g will lose approximately 0,09 oC for each gram of water it evaporates. Depending on the climatic conditions the amount of moisture loss will vary, but an average moisture loss during incubation is approximately 0,6 to 0,7% of the initial egg weight per day. This means that an egg of 60 g loses approximately 0.36 to 0.42 g of water per day, or 0.015 g to 0.018 g/hr.

Meijerhof and van Beek (1993) calculated the effect of moisture loss of eggs on temperature in different situations and at different positions in the egg. They calculated the temperature of an egg of 60 gram at the center and at the surface of the egg, 20 minutes after being placed from 40oC into 20oC. At the egg surface, the temperature of the egg was 29.74, 29.73 and 29.62oC, when moisture loss of the eggs was 0, 0.01%/h or 0.1%/h, respectively. At the centre of the egg, the temperature after 20 minutes was 35.17, 35.16 and 35.01oC, when moisture loss of the egg was 0, 0.01%/h or 0.1%/h. After infinitive time, the center temperature of the egg was 20, 19.96 and 19.56 with these amounts of moisture loss. As a normal moisture loss of an egg is approximately 0,6%/24 h or 0.025%/h, this means that although the effect on temperature is marginal, the egg shell temperature will always be slightly lower than air temperature, if no heat production by the embryo is present.

Positive heat production

During incubation the embryo starts to produce heat, as a byproduct of the metabolism of converting the egg content into body tissue. This heat production is noticeable from day 4 of incubation onwards, but it takes until day 8-9 before the machine has to start cooling to remove the heat, depending on the type of machine and eggs.

Heat production of eggs during incubation seems to differ between strains and breeds. The impression in the field is that modern broiler strains produce more heat than the strains that were used in the past. Part of this effect might be due to the fact that over time the average egg size has increased. As more body tissue is formed in a bigger egg, more heat will be produced as well. Also the egg composition might play a role in this, if the ratio albumen/yolk has altered over the years, which will also influence the heat production.

Some studies have shown differences in heat production between layer and broiler lines, (Nangsuay et al, 2011) and even within broiler strains within different breeds (Tona et al, 2010). Although this is a very intriguing area, one must be careful with the interpretation of the data. As Lourens et al (2007) showed, a difference in embryo temperature will result in a difference in heat production by the embryo, as its metabolism is altered. This means that incubation of eggs with equal air temperature but with different heat production will result in differences in embryo temperature and therefore differences in metabolism, which makes the heat production data difficult to quantify.

Temperature changes

To quantify the effect of the environment on the temperature of the eggs, one can work with the concept of half times. A half time is defined as the time a body (an egg) needs to cover half of an initial temperature difference. This means that if an egg is placed from 40oC into 20oC, the first half time is the time the egg needs to cool from 40oC to 30oC, the second half time is the time needed to cool from 30oC to 25oC etc. This method allows to compare different temperature profiles, as the half time will be more or less equal for each temperature difference, and also the first, second and consecutive half times are comparable. It is normally assumed that a body has a constant temperature after 5 half times. Although there is a slight difference in half times between cooling and warming bodies, for practical purposes this difference can be neglected. The theoretical method to calculate half times in spherical bodies is presented in Meijerhof and van Beek (1993), but at a low air velocity of 0.2 m/s, an uncovered egg will have a half time of approximately 30 minutes. This is highly dependent on the way of storing the eggs, as an egg in the centre of a stack of eggs on cardboard trays will have a half time of several hours.

Air velocity

Air velocity has a major impact on heat transfer, and with it on the half time of eggs. If there is no heat production in an egg and no difference in temperature between egg and air, air velocity has no effect, simply because the egg is not cooling or heating and therefore has no half time to experience. Cooling or warming of eggs goes much faster if there is a significant air velocity over the eggs. If air velocity is increased from 0 m/s to 0,5 m/s, the heat transfer and with it the cooling or warming rate of eggs is increased approximately 4 times (Meijerhof and van Beek, 1993). A further increase to 2 m/s has relatively limited added value, as at this air velocity rate the heat transfer is 5 to 6 times higher than at 0 m/s.

Heat transfer during incubation

During incubation, the embryos start to produce increasing amounts of heat, which will increase the embryo temperature above air temperature (Sotherland and Rahn, 1987) if not adequately removed to keep the embryo at the desired temperature (French, 1997). At a given heat production of the embryo, the temperature of the egg will rise above air temperature, depending on air temperature and air velocity. Meijerhof and van Beek (1993) showed that with a heat production of 3 W/kg egg (0,18 W/egg at an egg weight of 60g), the temperature difference between egg and air would be 0,75oC for an egg of 60 g in an air velocity of 2 m/s, but 1,4oC for the same egg when placed in an air velocity of 0.5 m/s. With increasing egg weight (at equal heat production/kg) the differences in temperature between egg and air increase further. Eggs of 75g with the same heat production per kg were calculated to be 1.7oC warmer than air temperature at an air velocity of 0.5 m/s, and 0.8oC warmer at an air velocity of 2 m/s. The reason for this effect is that a bigger egg has more heat production per egg, but also more egg content per surface area, which limits the heat transfer. These theoretical results indicate not only that for an uniform embryo temperature in an incubator the air velocity over the eggs need to be uniform at all places, but also that it is beneficial to have a relative high air velocity to equalize differences between egg sizes in one machine.

Lourens (2001) measured in a field situation the egg shell temperature of eggs after 18 days of incubation and placed in 37.2oC air temperature. He found that at an air velocity of 0 m/s, the egg shell temperature was 40.3oC, where at 0.5 m/s the egg shell temperature was only 38.4oC. When the air velocity increased further to 2 m/s, the egg shell temperature dropped to 37.7oC. These

results confirm that a small increase in air velocity in the lower range of air velocity has more effect than increases in the high air velocity ranges.

A complicating factor with the interpretation of practical measurements is the effect that temperature by itself has on embryo metabolism and therefore heat production. Lourens et al (2007) and Molenaar et al (2010) showed that an increase in egg shell temperature of 0.9oC resulted in an increase in heat production until incubation day 15, but a decrease in heat production later in incubation. At day 18 and 19, the higher embryo temperature resulted in approximately 10% less heat production. This means that in experiments with factors that have an influence on heat transfer or heat production, the possible influence on embryo temperature and with it on embryo development need to be evaluated. Especially experiments that can influence air velocity over the eggs, for instance experiments with turning angle or turning frequency need attention.

Relative humidity

During the developmental process, embryos produce metabolic water (Romanoff, 1960). As the embryo needs an air cell to initiate lung respiration, this metabolic water needs to be lost during the incubation process. The effect of moisture loss on incubation results has been widely studied, and its general accepted that optimal moisture loss until internal pipping is approximately 12 to 14% of the initial egg weight. During the incubation process, CO_2 is expelled from the egg and O_2 is taken up which have a different molecular weight, but due to the RQ value of respiration the change in egg weight can be fully explained by the moisture loss of the eggs (Ar and Rahn, 1980). This means that weighing the egg at different moments is adequate to determine moisture loss.

Moisture loss of of an egg is related to the driving force for moisture loss and the porosity or conductance of the shell. For the driving force, several properties can be used: water potential (Pa), water vapor concentration (kg/m3) water vapor pressure (Pa) and mol fraction (mol/mol). Theoretically, the best property to use is the mol fraction as the related coefficient of diffusion of water vapor only depends on temperature and is independent of pressure (Nobel, 1983). However, using water vapor pressure is more commonly used. Moisture loss of eggs is often predicted with Fick's first law of diffusion (Ar et al, 1974), and depends on the resistance of the egg shell and membranes and the difference in water vapor pressure across the egg shell. Fick's law is based on egg shell conductance expressed as milligrams of water per Torr per day. The author prefers to calculate with a transpiration coefficient based on SI units, which allows to use the Mollier diagram to calculate water vapor pressure deficits under variable situations, including warming and cooling of eggs (Meijerhof and van Beek, 1993).

Air velocity does not have a direct effect on moisture loss (Meijerhof and van Beek, 1993). This is due to the fact that the limiting factor for moisture loss is the passage of moisture through the shell, and no the removal of water from the surface of the shell. As air velocity influences only the release of water from the shell surface, moisture loss is a function of water vapor pressure deficit and conductance, which are not influenced by air velocity.

The water vapor pressure difference across the egg shell is determined by temperature and relative humidity inside and outside of the egg. Because the of the high water content, the water vapor

pressure in the egg is nearly saturated, which means that the water vapor pressure can be calculated for absolute humidity with the Magnus equation, and will only be dependent on temperature. The water vapor pressure outside the egg is a function of temperature and relative humidity of the air surrounding the egg. As temperature inside and outside of the egg are relatively constant, it means that relative humidity of the air is the most important factor to control moisture loss. The amount of moisture loss will be related to the change in water vapor pressure in the air, which will be dependent on relative humidity (at a given temperature) and can be obtained from the Mollier diagram. However, it must be realized that also temperature changes of for instance the egg shell have an impact on moisture loss. This means that if embryo temperature changes, a slight change in moisture loss will be observed. This is the reason why sometimes air velocity seems to influence moisture loss, as it influences the heat transfer of the eggs and therefore its temperature, which will change the water vapor pressure deficit over the shell.

The other factor that determines moisture loss is the conductance of the egg shell, basically the resistance of the shell against passage of gases. The conductance is the combined effect of the resistance of the shell membranes, the number, shape and size of the pores in the egg shell and the added effect of the cuticle. During incubation the embryo utilizes calcium from the inside of the egg shell for bone formation, but this doesn't influence the resistance of the egg shell against gas exchange. In normal situations the conductance of an egg will not change during storage or incubation, unless the cuticle of the egg is removed. This can happen when eggs are washed or sprayed with certain detergents. The conductance can artificially be lowered by for instance spraying oil over an egg shell, as is sometimes practiced with table eggs to increase their shelf life. The conductance of an egg can be measured by the weight loss over time, if the water vapor pressure deficit over the egg shell is known. If the conductance of an egg is expressed as a percentage of weight loss per time unit per Pa of water vapor pressure deficit (kg/(kg.Pa.s), we can actually predict moisture loss of that egg in every situation if we know the temperature and relative humidity (and therefore the water vapor pressure), by measuring the weight loss of an egg over time in any situation with a known water vapor pressure. So if we measure weight loss during storage or the first days of incubation (with known temperature and relative humidity) we can simply predict the total weight loss of these eggs over the total incubation period, and we can adjust the relative humidity to the required water vapor pressure deficit if the predicted value for moisture loss doesn't meet the requirements.

Several researchers have found that the optimum for moisture loss until pipping is between 12 and 14% of the initial egg weight (Meir et al, 1984, Hulet et al, 1987). This can be controlled rather accurate with relative humidity if the conductance is known. However, in nature the moisture loss of eggs will be very variable as the incubating bird has limited possibilities to influence the relative humidity in the nest. Neither can they change the conductance of the eggs based on the expectations of weather for the coming period. Visscherdijk et al (1985) demonstrated a high variation in conductance between individual eggs, as high as 20%. Tullett (1981) has shown that individual eggs can tolerate a high variation in moisture loss before their hatchability is affected, which is in agreement with the fact that birds cannot actively influence the moisture loss during natural incubation. The apparent contradiction between a limited range for optimum moisture loss and a wide range of acceptable moisture loss for individual eggs is can be explained by the high variation in moisture loss between individual eggs. Due to this high variation, the average moisture loss of the

eggs in the machine must be between rather narrow limits, to avoid extreme moisture losses of individual eggs that will affect their chance on successful hatching.

Gas exchange

Besides water vapor, also oxygen and carbon dioxide needs to be exchanged over the egg shell. This gas exchange is also dependent on the conductance of the egg shell, as the exchange has to go through the same pores as the water vapor. At the moment of production, the egg contains carbon dioxide equal to the blood level. After oviposition, carbon dioxide is released from the egg content which forces the pH of the albumen to rise in a few days to a level of approximately 9.3-9.5. This helps to protect the egg against micro-organisms and has a function in early development of the embryo (Stern, 1991). When incubation starts, the embryo slowly starts to produce carbon dioxide due to its metabolic activity, which forces the pH to decrease again (Gillespie and McHanwell, 1987). Until internal pipping, these gases are brought to the shell by the blood stream from the embryo towards the shell. In natural incubation conditions, this blood stream is influenced by the temperature of the egg (Tzschentke, 2007; 2008), as the embryo uses the gradient between bottom of the nest and brood path of the hen to regulate its own internal temperature. If this has an effect on the gas exchange of the embryo is not known.

It is well known that incubation at higher altitudes (higher than 600-700 meter above sea level) increases embryonic mortality numbers and decreases hatch, due to the reduction of the partial pressure of oxygen. Because incubating embryos respire by diffusion, the diffusion rate and with it respiration decrease with barometric pressure reduction. Bagley and Christensen (1989) showed that reduced diffusion rates reduced organ maturation and body weight. Lokhorst and Romijn (1965) already reported a reduction of hatchability and of heat production when eggs were incubated at increased altitude. Incubation at a simulated altitude of 3000 m above sea level reduced the heat production of the embryos with almost 50%, and embryos did not hatch anymore.

Lourens et al (2007), incubated eggs at 17, 21 and 25% oxygen concentration, and at an egg shell temperature of 37.8oC (normal) and 38.9oC (high). Until day 15, eggs incubated at the higher egg shell temperature produced more heat than eggs incubated at the normal profile. At day 16-17, eggs incubated at the higher egg shell temperature profile in combination with the lower oxygen concentration started to produce less heat than the other groups. At day 18 and 19, only the eggs at 25% oxygen concentration continued to produce more heat when incubated at the higher egg shell temperature. This suggests that there needs to be a balance between the speed of the development of the embryo, which is determined by the temperature in the egg, and the availability of oxygen for the embryo. Oxygen availability for the embryo is dependent on the altitude above sea level and oxygen concentration in the air, but also on oxygen conductance of the egg, on blood cell formation of the embryo, and on ability to release carbon dioxide from the egg to the surrounding air and therefore on carbon dioxide concentration in the machine. This suggests that it might be beneficial to adjust embryo temperature to the availability of oxygen for the embryo, which needs further study.

In single stage incubation, ventilation can be adjusted to the stage of development of the embryos. Nowadays, ventilation is often closed completely for the first several days of incubation, which increases the carbon dioxide levels. It is shown that this higher level of carbon dioxide results in an increase of blood vessel development early in incubation (Ognabesan, 2007). The effect of this on embryo development, hatchability and chick quality needs to be further examined.

In single stage incubation, often CO2 levels of 3000-4000 ppm are used in the second half of the incubation process, to reduce the amount of ventilation. In multi stage incubation levels do not often exceed 1500 ppm, due to the fact that often in these machines the cooling capacity is limited, and more air exchange needs to be used for cooling. During the final stages of incubation and hatching, high levels of CO2 stimulate the pipping and hatching process and shortens the period between internal and external pipping (Visschedijk, 1968). In field situations, carbon dioxide levels of 4000-5000 ppm are often maintained during the hatching stage, as it stimulates the hatching process and makes the difference in hatch time smaller. This is due to the fact that high levels of CO2 stimulate the hatching process, but also because a high level of CO2 means a limited amount of ventilation and with it a more uniform distribution of embryo temperature. The interactions between parameters as ventilation and temperature are complex but important to understand the balances in environment that occur in the machines.

Interactions between parameters

One of the biggest challenges involved in the physical conditions during incubation are the interactions between different parameters, and then especially the effect that some conditions can have on temperature of the embryo. This makes the interpretation of the effects of that specific adjustments in parameters have complicated, especially if egg shell temperatures are not controlled or adjusted.

As discussed, embryo temperature during incubation is the result of the heat production of the embryo and the heat loss from the egg to the environment. Lourens et al (2007) showed that at the end of incubation, heat production of the embryos decreases with increasing embryo temperature and decreasing oxygen availability. This means that a change in environment resulting in a difference in heat loss might change the heat production of the embryo, although the direct influence of the change on the embryo might be limited. Lourens et al (2006) showed that the difference in heat production and development of embryos in big and small eggs is proportional to their difference in egg size, when both groups are incubated at the same egg shell temperature. However, if these eggs are incubated at the same air temperature, egg shell temperature of the bigger eggs will be higher than that of the small eggs due to differences in air velocity over the eggs and shell/surface ratio's. Due to the differences in embryo temperature, differences in embryo development and heat production per gram of egg can be expected.

Especially air velocity over eggs can change rapidly when difference in turning, tray position, egg size, ventilator speed etc are applied. As air velocity has a high effect on heat loss and therefore on egg shell temperature, experiments with for instance turning should always be done with a strict control of embryo temperature, to avoid misinterpretation of results.

The level of ventilation and the condition of the air that is used for ventilation can have a significant influence on the heat balance during incubation, and with it on the temperature of the air and the eggs. The amount of ventilation and the temperature of the air that is brought into the machine influences the heat exchange of the machine. But as relatively cold air is brought into the machine, the machine has to add water to maintain the relative humidity at the often used level of 50-55%. This water requires energy to evaporate, and with it is functions as a major cooling force, although an uneven distribution of the water spray throughout the machine can lead to local cool spots.

Especially if cooling capacity of machines is limited, it is important to avoid fluctuations of the amount of spraying provided, to ensure a climate in the machine that is as even as possible. In multi stage machines, the ventilation rate is more or less constant over time, which means that only a change in condition of the air will influence the amount of spraying that is provided. This makes it important to control the condition of the air in the setter room, to avoid unexpected operation of the sprayer. In single stage machines, the amount of ventilation is adjusted during the incubation process, to facilitate the eggs with sufficient air for respiration. The increased amount of air that is needed at the end of the incubation process requires an increasing amount of spraying, to compensate for the increasing amount of relatively dry air entering the machine. To avoid the fluctuating amount of spraying and with it a relative imbalance of the machine, the concept of non-linear moisture loss is often used. With this concept, a higher level of relative humidity (and consequently a lower level of moisture loss by the eggs) is used in the first period of incubation. Over time, when ventilation is increasing, the level of relative humidity is lowered (and moisture loss of the eggs increased), and with it the amount of spraying that needs to be used is decreased. As the main function of the moisture loss is to create a sufficient air cell at the moment of internal pipping, non-lineair moisture loss functions well as long as the total required moisture loss is achieved.

As ventilation rate influences CO_2 levels in the machine, changing the CO_2 level by opening or closing the damper wil not only influence CO_2 but also the relative humidity of the machine, and with it the amount of cold air entering and the amount of spraying needed to maintain the relative humidity. As this might influence the heat balance and with it the embryo temperature of the eggs, the result of a change in results after changing the CO_2 level might sometimes be caused by a change in temperature instead of being a direct result of the change in CO_2 itself.

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